Assurance of physical equivalence (size, charge, concentration, aggregation, interactions) of complex nanomedicine substances

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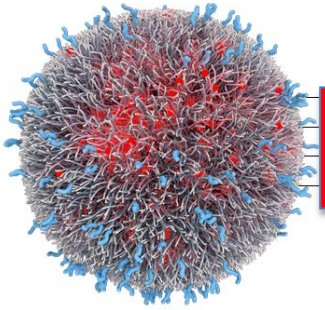
Context:

- Nanomedicine regulation
  - Nanoparticle based delivery of generics
  - Nanomilled APIs
  - Vaccines
- Innovation will provide medical and cost benefits, better ways of using existing drugs
- Safety and certainty are still required

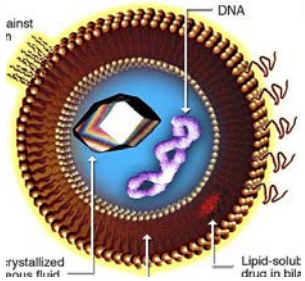
Rapid development and use of complex nanomedicine products

- From the lab
- To Market
- To Generic

BIND THERAPEUTICS
A new way of targeting disease



Alnylam PHARMACEUTICALS



HARVARD

LIQUIDIA TECHNOLOGIES

Johnson & Johnson

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Ben Venue Laboratories

SUN PHARMA

AZAYA THERAPEUTICS

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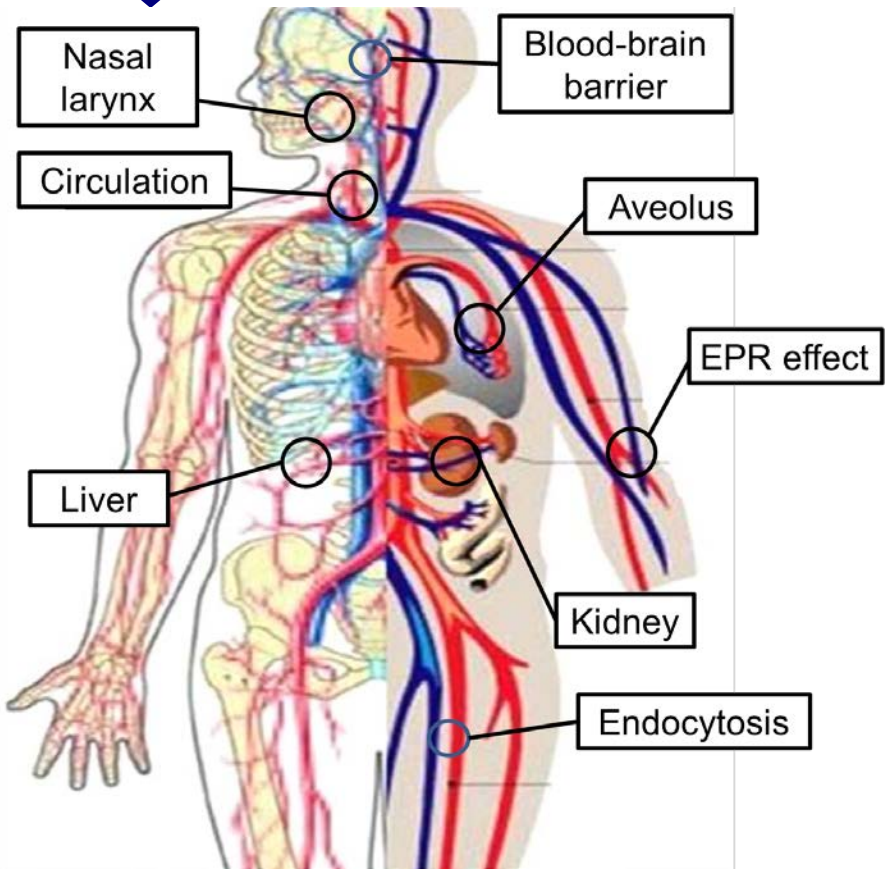
Importance of the physiochemical properties of drug delivery particles



Concentration

- Drug delivery dosage
- Disease stage/progression
- Toxicity

Size

- Localized accumulation

  Nasal, liver, kidneys, EPR effect, blood-brain barrier,
- Effluent pathway
- Toxicity

Surface Charge (ζ-potential)

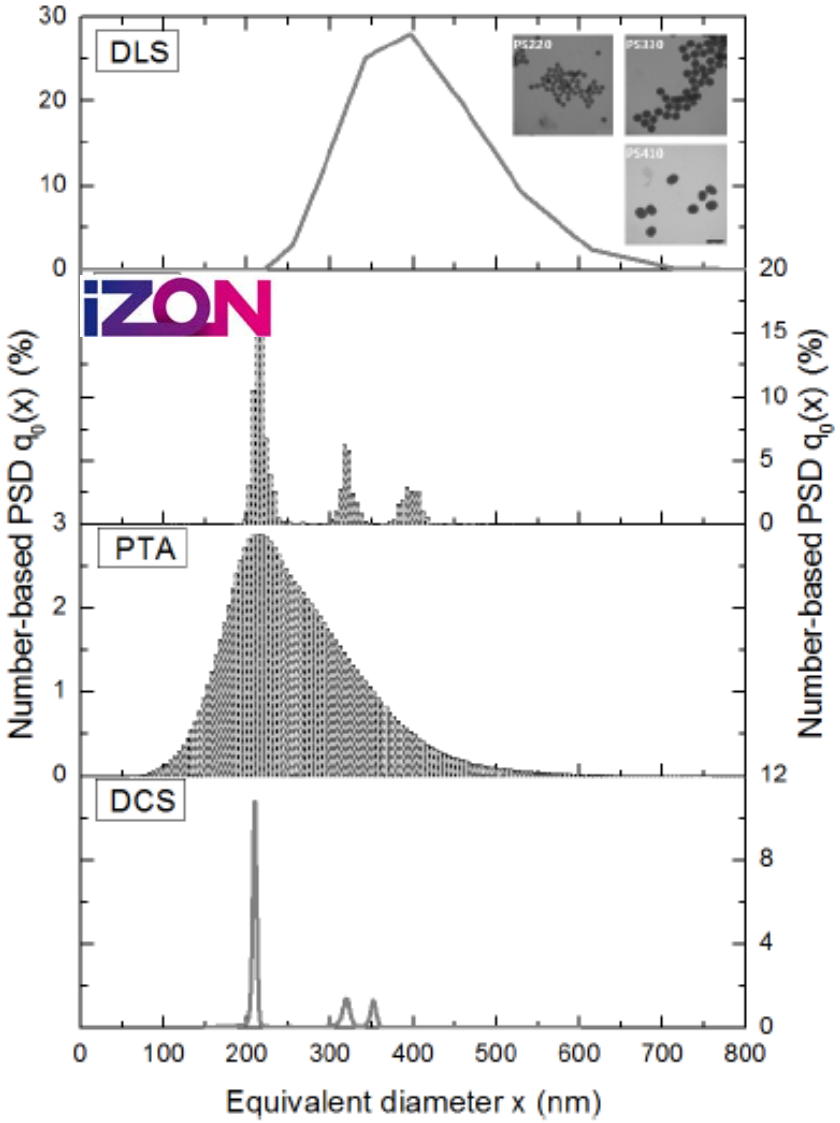
- Endocytic mediated pathway
- Toxicity

Need for accurate and sensitive analysis techniques that measure the properties of each particle in physiological conditions

Proposal:

- Demonstrate physical equivalence by accurate, precise and repeatable suite of particle measurements
- “Particle fingerprinting” (previous speaker’s proposal)
- This would replace present method of requiring no change to manufacturing process, which is not practical in scale up situations
- Needs to be practical and cost effective

Long established techniques have insufficient resolution for nanomedicines



Dynamic Light Scattering is the most common particle analysis technique but lacks resolution and repeatability

Particle-by-particle **"number-based"** distributions can now provide a more realistic and accurate measure of particle size distribution

New techniques offer solutions but need FDA support

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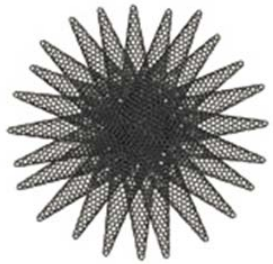
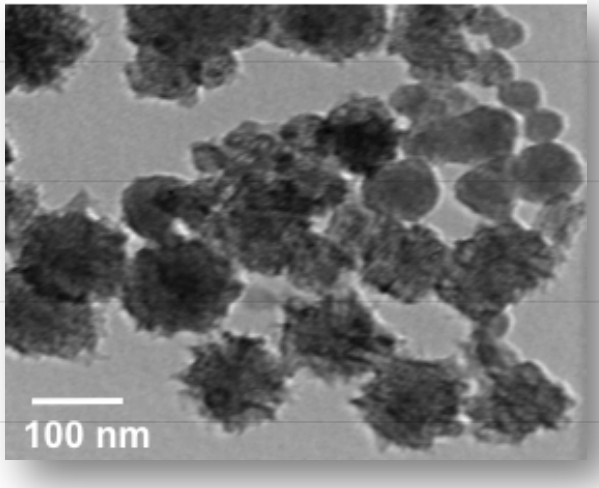
TRPS vs DLS vs EM of complex particles: Carbon Nanohorns





TRPS
DLS

% Number (DLS)

% Population (TRPS)

Size (nm)

0 75 150 225 300 375 450 525 600 675 750 825 900 975

0 2 4 6 8 10 12 14

0 2 4 6 8 10 12

Possible Suite of Measurements:

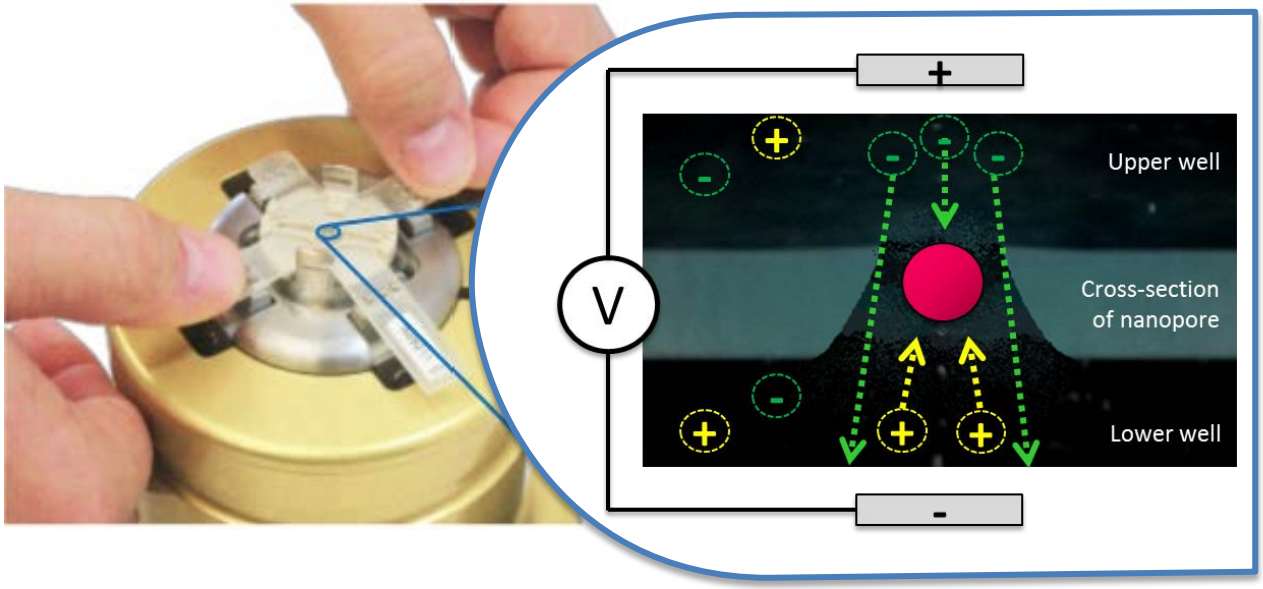
- Particle number (concentration)
- Accurate, calibrated size distribution based on particle by particle analysis
- Particle by particle surface charge (charge affects behaviour as much as size)
- Particle interactions, including aggregation and biomolecule interaction

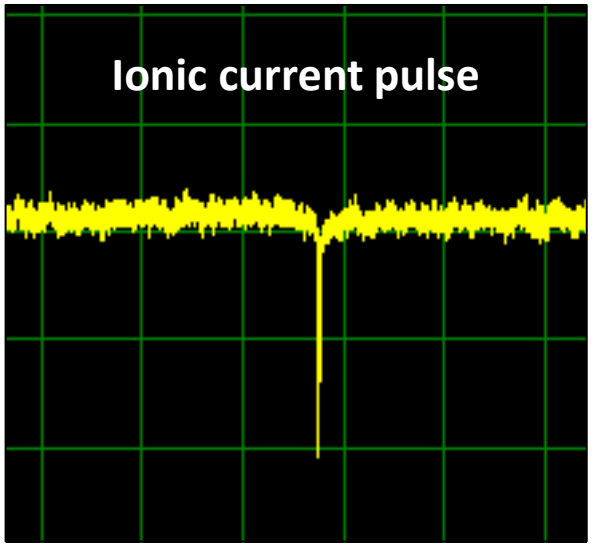
Nanomedicine examples

- Liposomes,eg Doxil
- Emulsions
- Bioparticles
- Virus like particles (VLPs)
- Polymer capsules
- NP based vaccines
- Virus based vaccines

TRPS – Single Particle Analysis

When a particle enters the pore it blocks a proportion of the ions flowing through the pore – this causes a pulse in the ionic current the magnitude and duration of the pulse is proportional to the particle size and velocity, respectively.





Particle-by-particle measurements provide high resolution analysis

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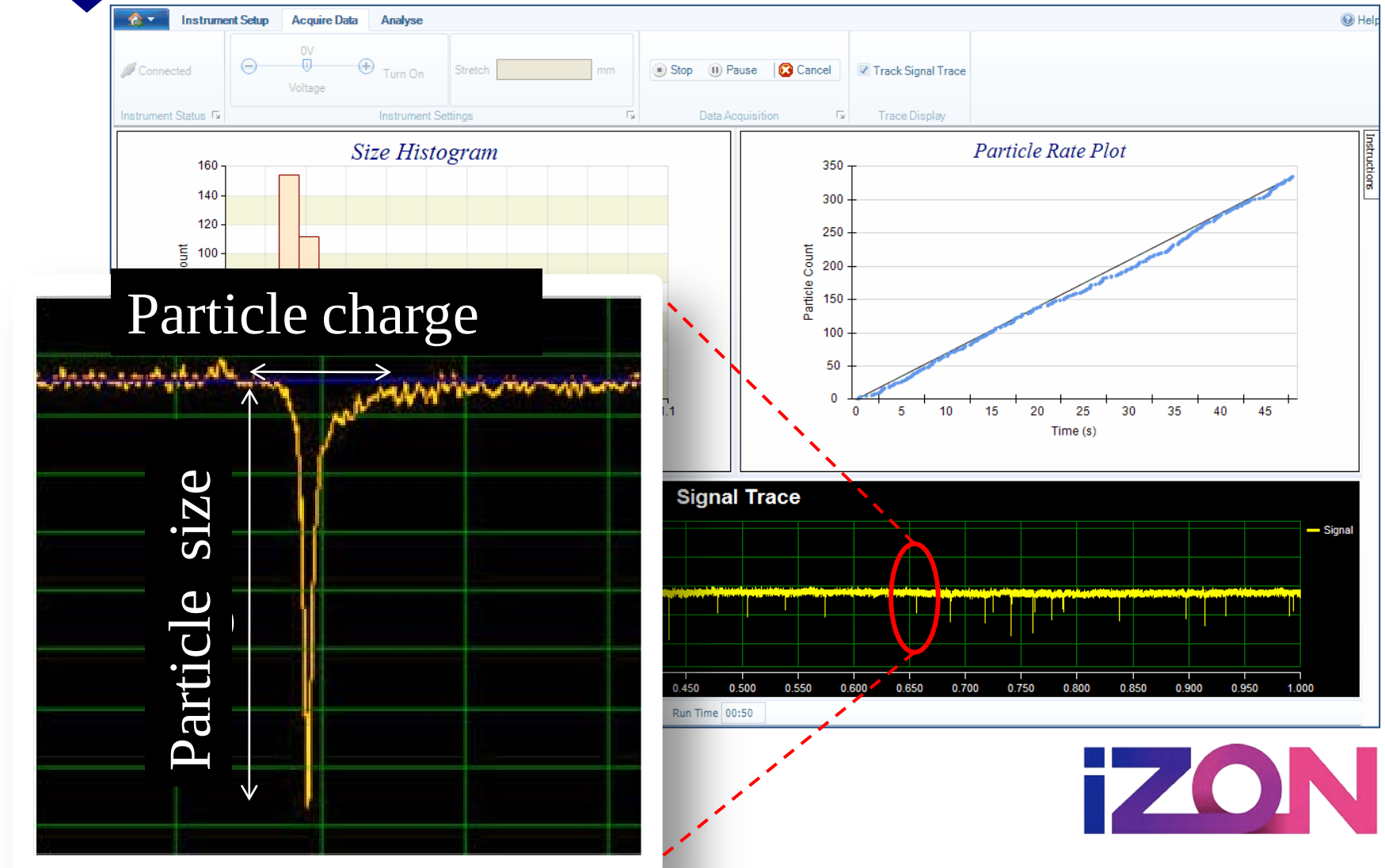
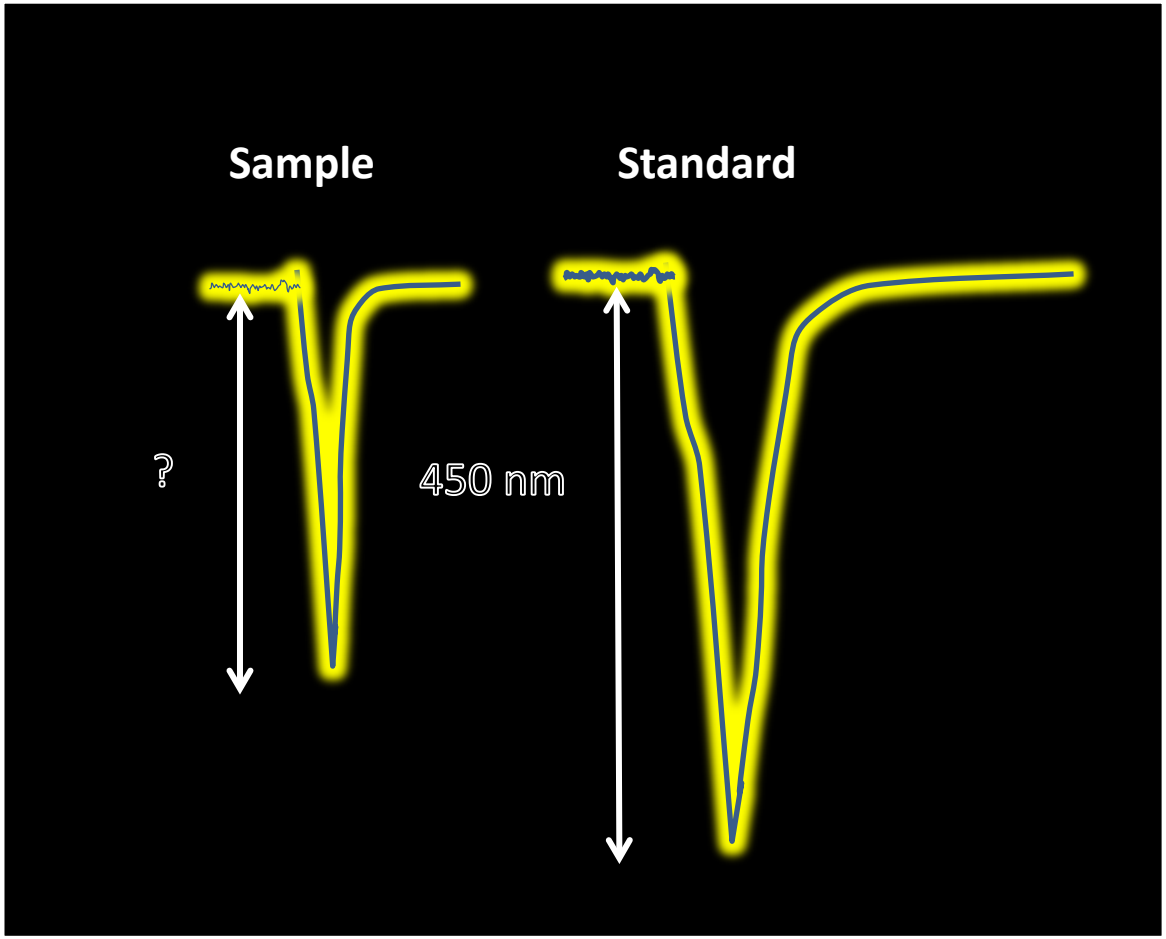
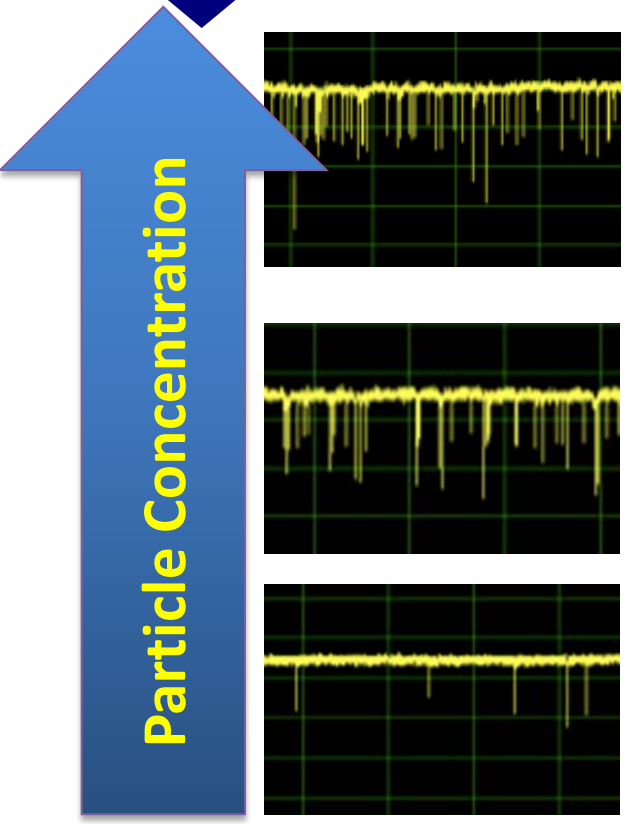
TRPS Principles

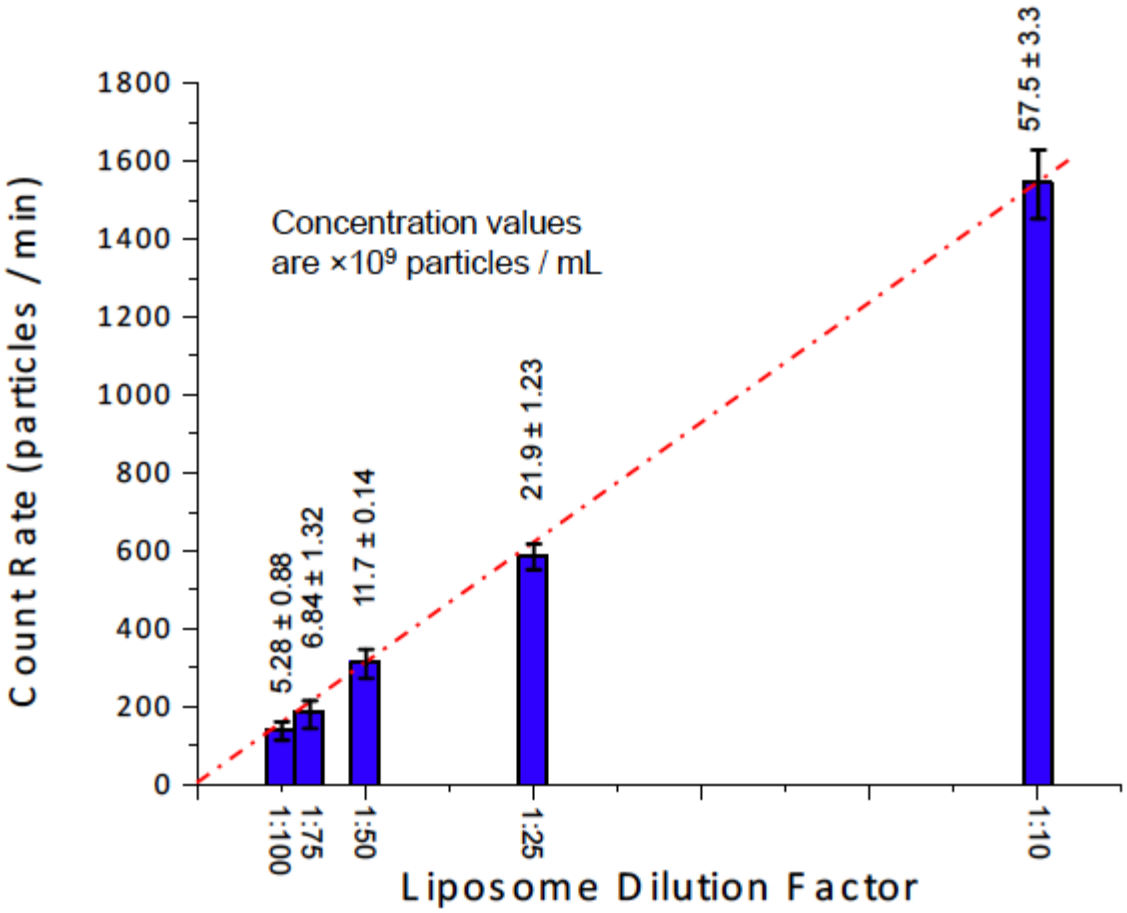


Assurance through calibration



Particle Rate ∝ Concentration

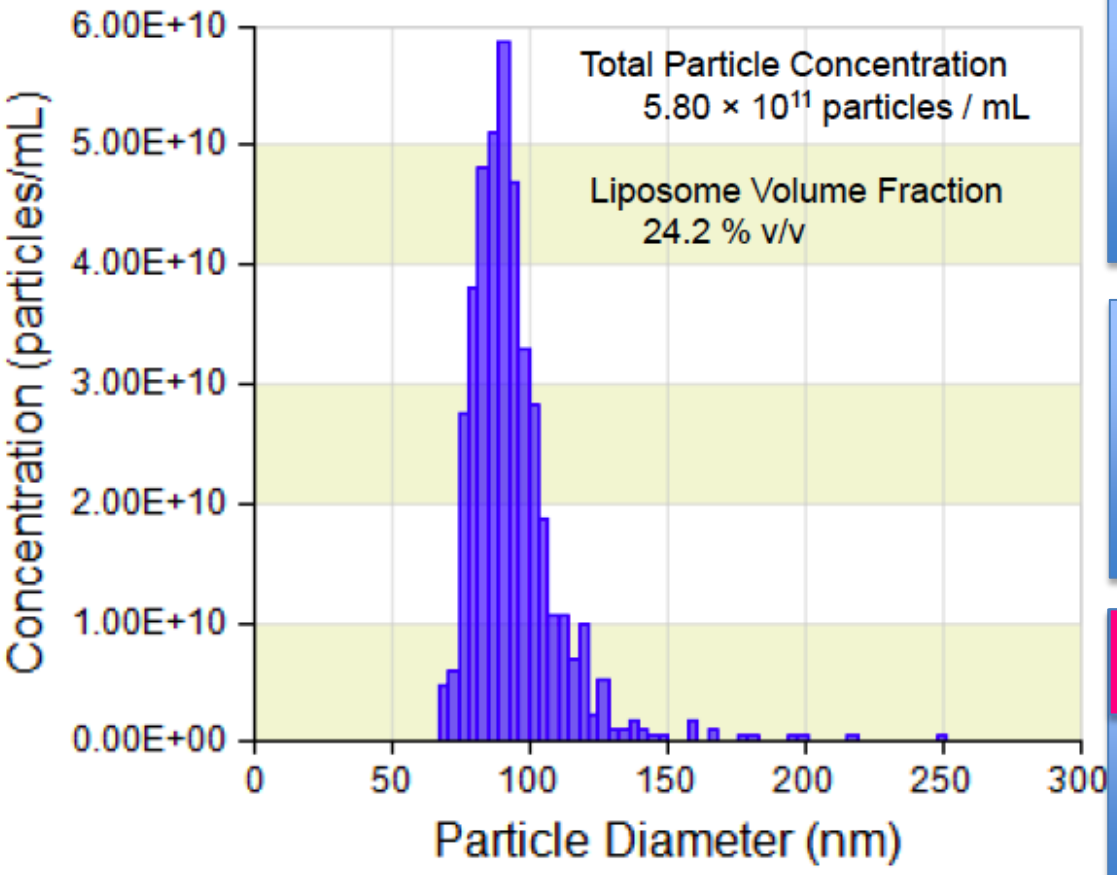


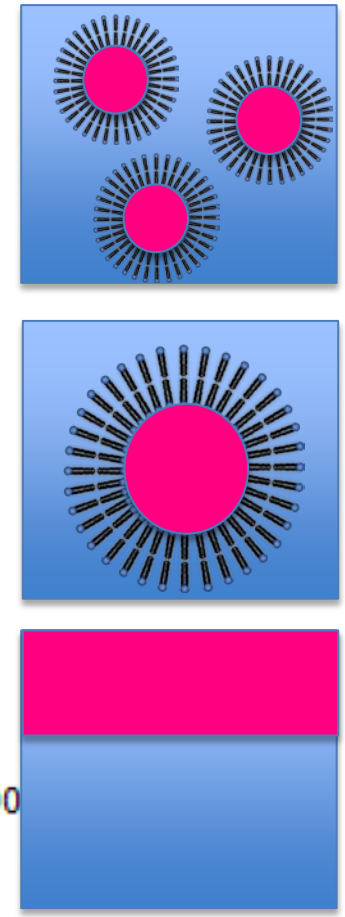


Roberts *et al.* (2012) *Biosensors and Bioelectronics* 31 pp. 17-25

Size-specific concentration

Measuring the properties of each particle and their concentration enables the deliverable liposome volume fraction "dosage" to be calculated



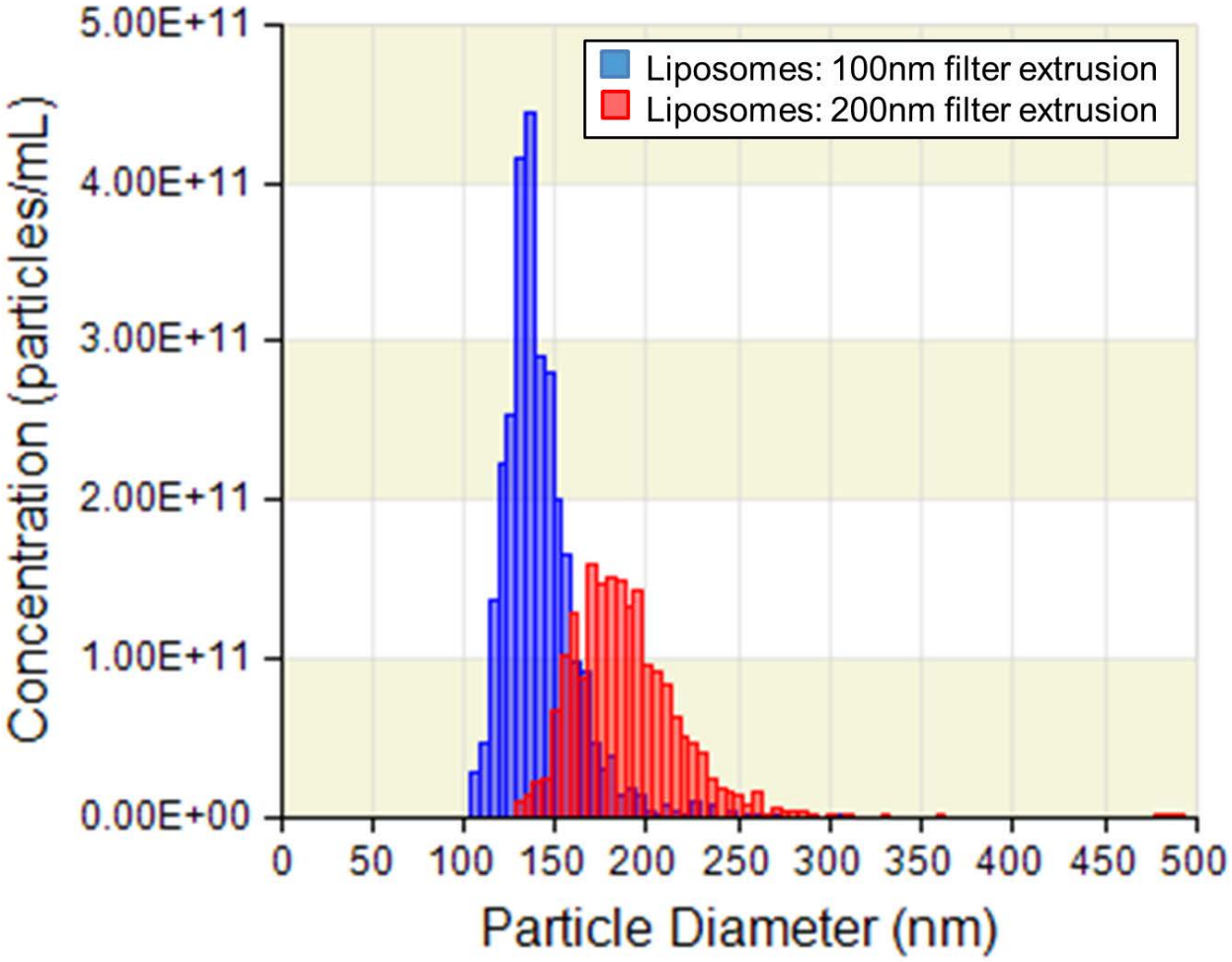


Liposome volume fraction

Particle volume $4/3\pi r^3$ (size)

Particle number (concentration)

TRPS Size Analysis for Optimizing Liposome Synthesis



High resolution means confidence and precision in tailoring the size of all the liposomes in solution.

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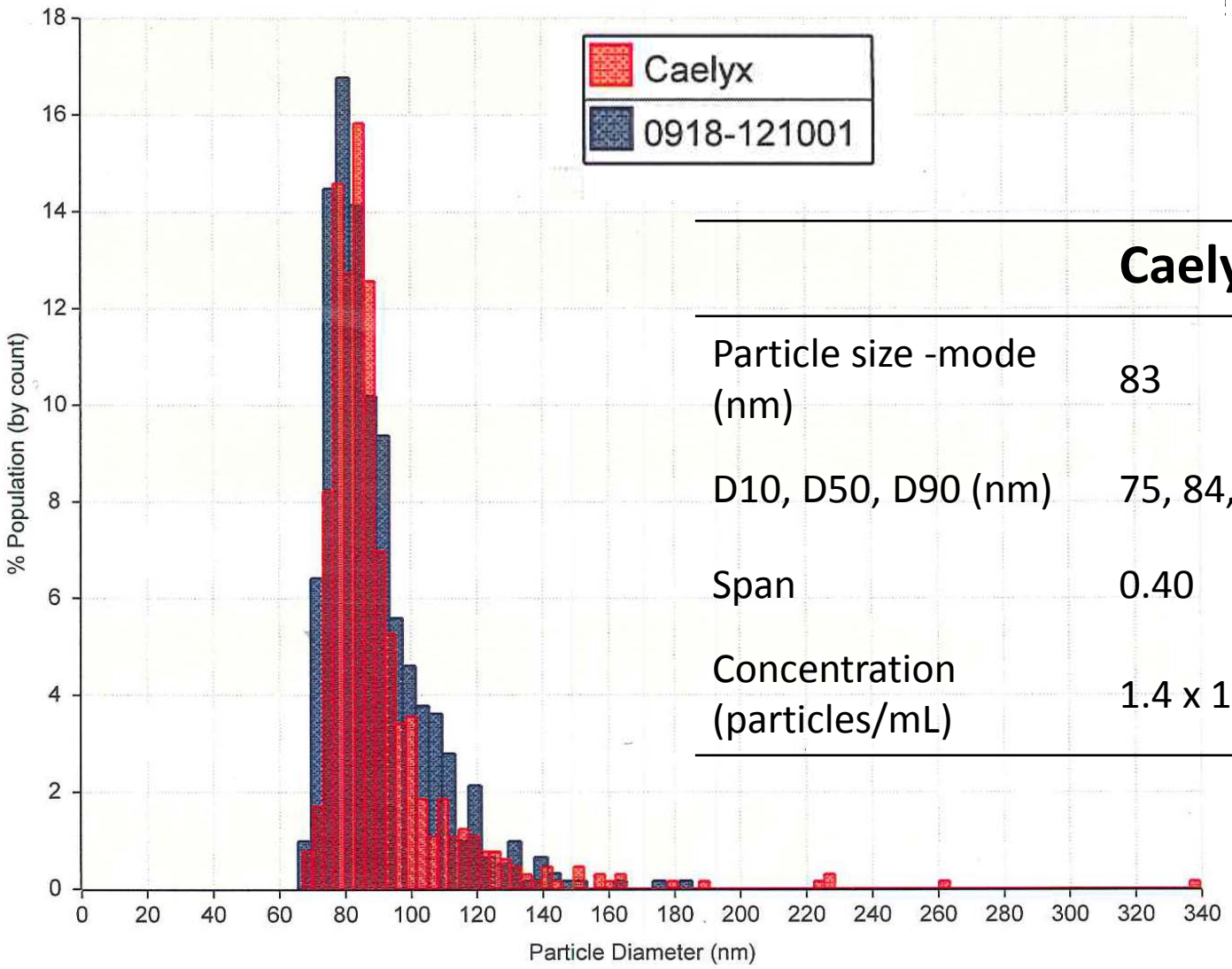
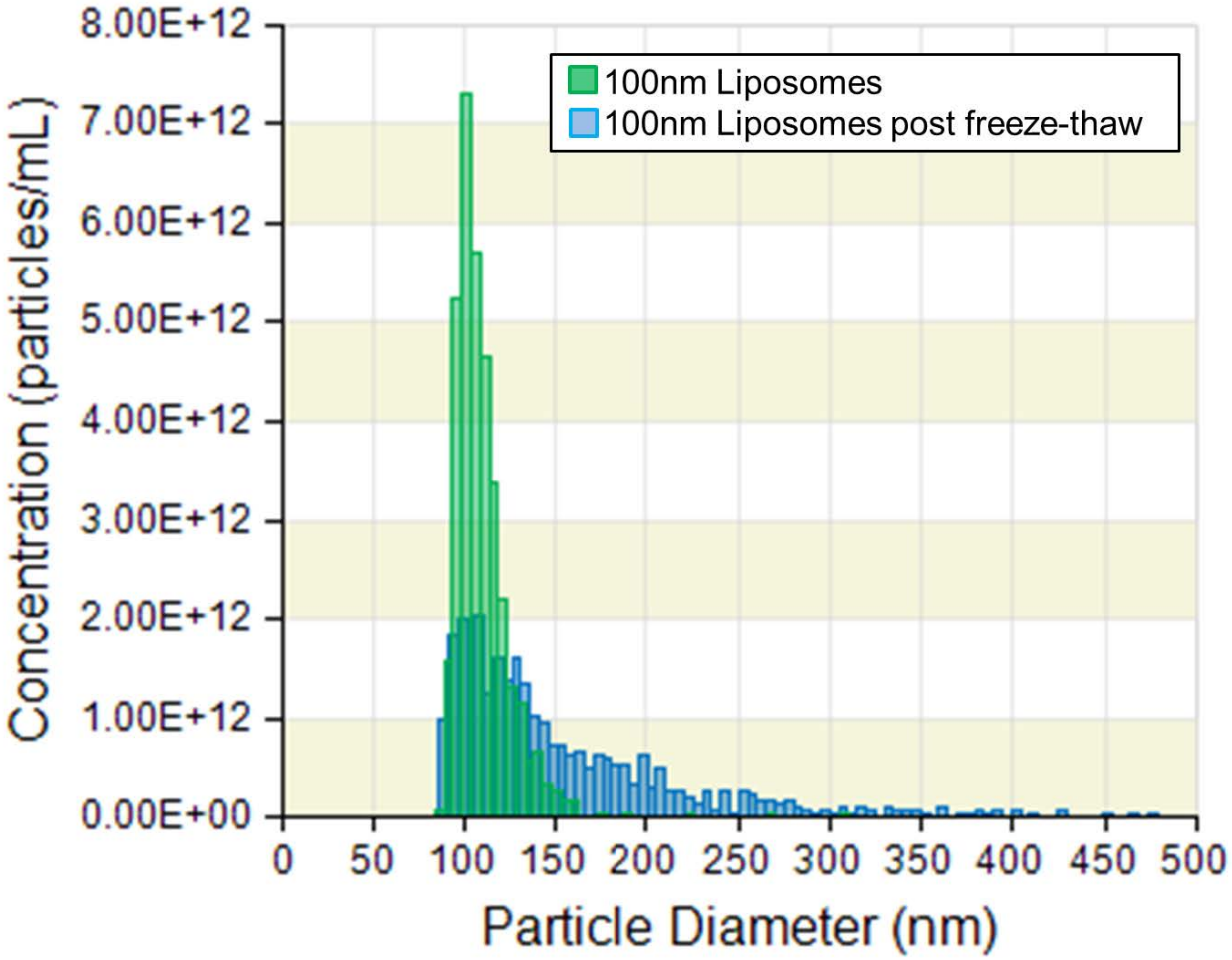
Caelyx vs a generic



	Caelyx	Generic
Particle size -mode (nm)	83	78
D10, D50, D90 (nm)	75, 84, 109	73, 83, 108
Span	0.40	0.42
Concentration (particles/mL)	1.4×10^{12}	1.7×10^{12}

TRPS Size Analysis for Monitoring Liposome Stability



High resolution means confidence in understanding the affects of treatment and storage conditions of long-term stability of liposome solutions.

Field based measurement may be required

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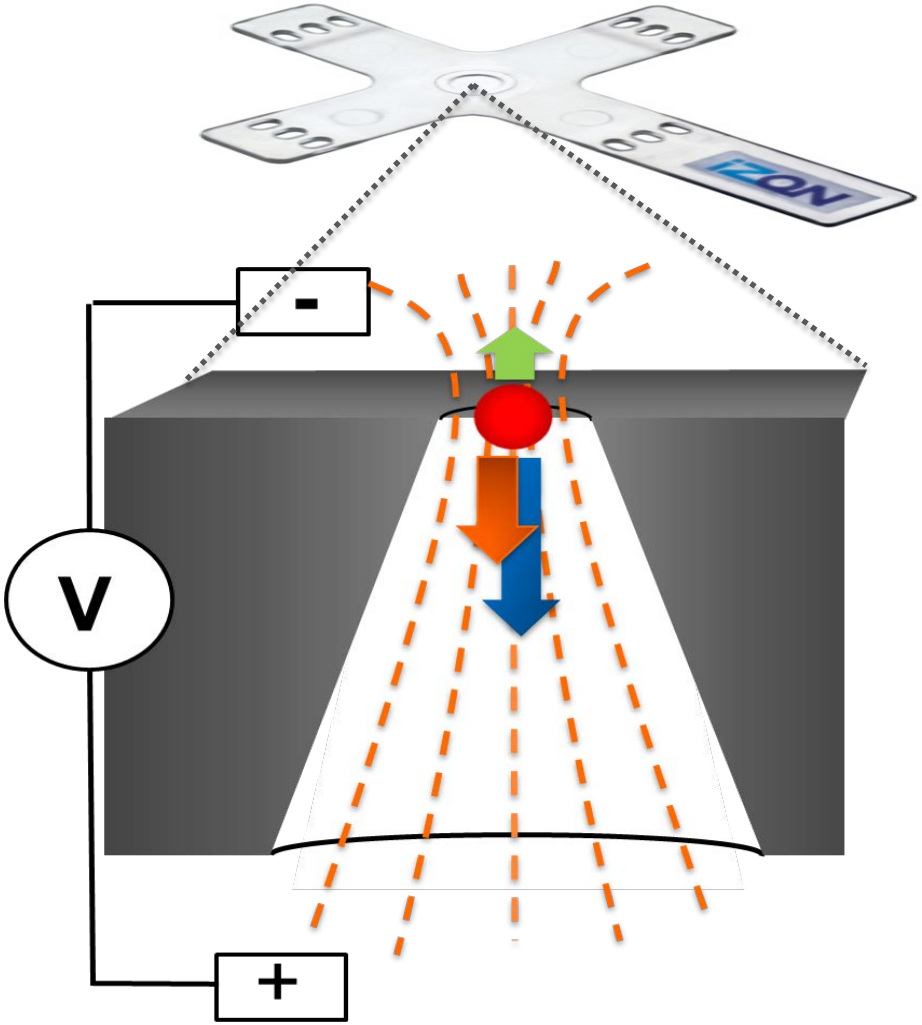
Using Izon instruments to measure particle charge



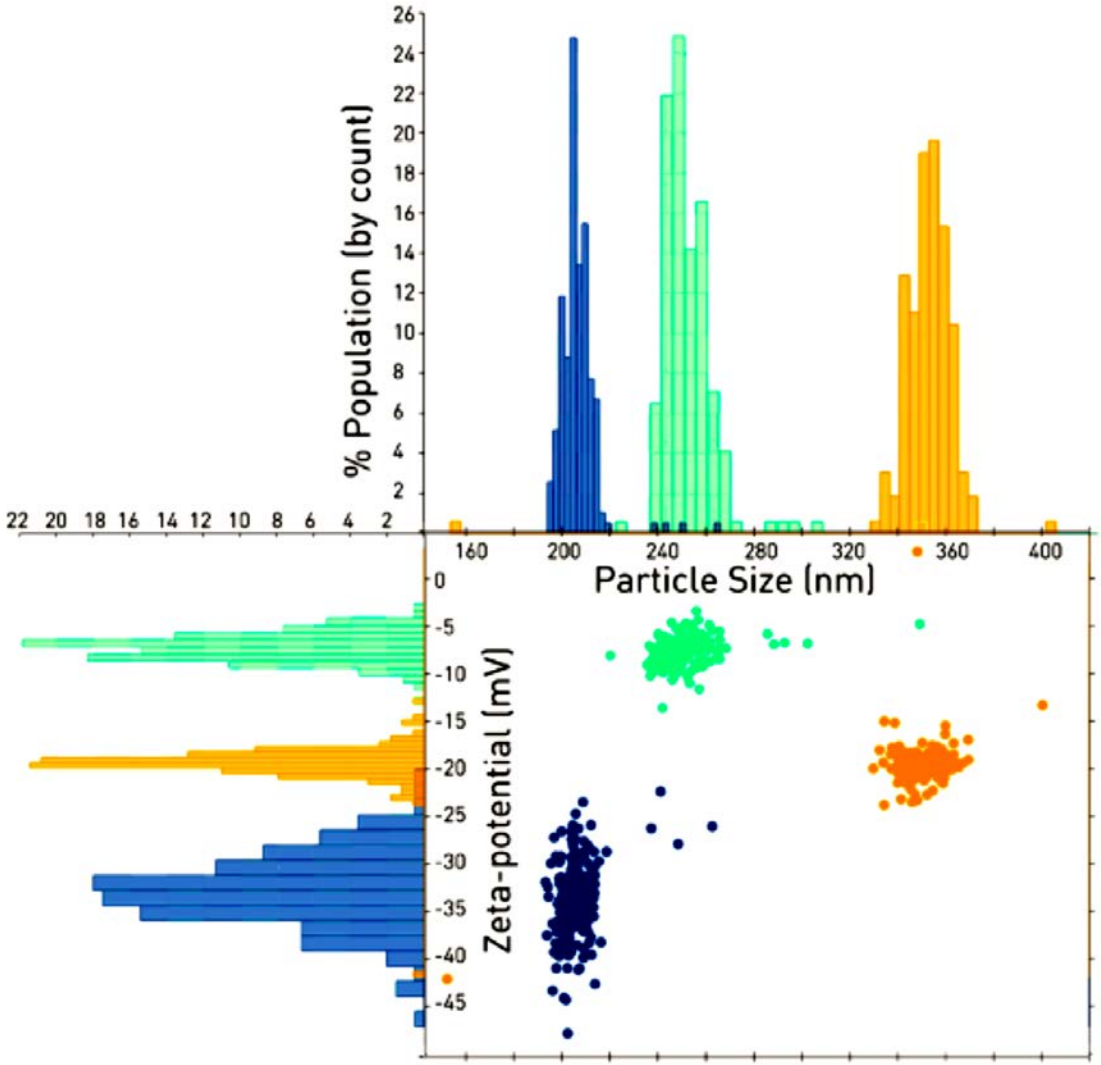
- **Particle velocity through the pore is due to:**

Fluid Velocity (convection)
Directionally dependent and proportional to the applied pressure.

Electrophoretic Mobility
Proportional to particle charge (ζ-potential) and applied voltage.

Electro-osmosis
Proportional to pore charge (ζ-potential) and applied voltage.

High Resolution Particle-by-Particle Size & Charge Analysis



Represents a new way and ability to characterize and understand particles

Each dot represents a single particle and histograms illustrate the distribution

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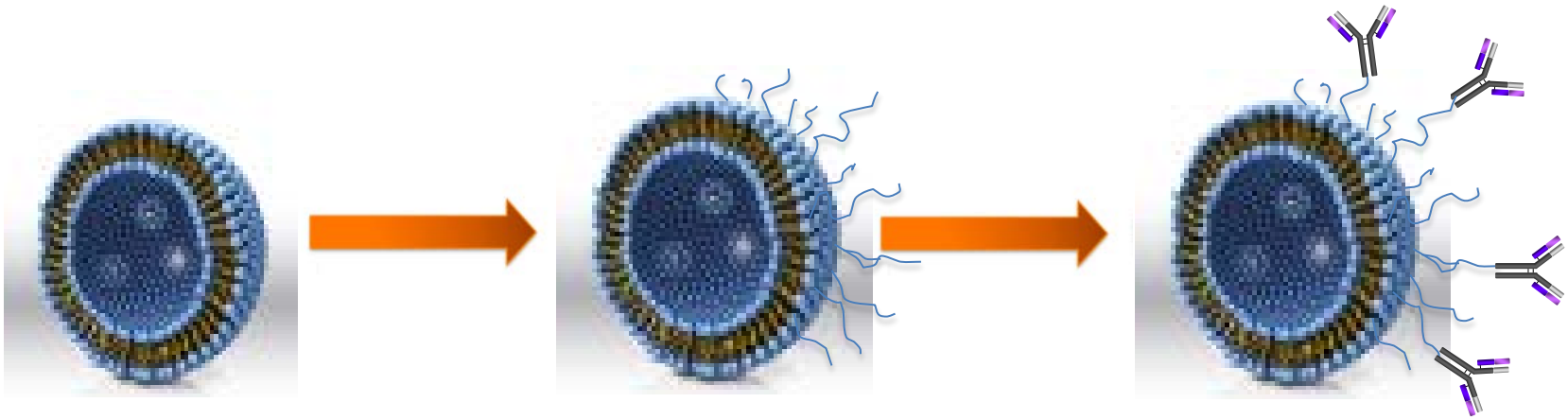
Size & Charge Analysis of Liposomes

Modifying the surface of liposomes is used to improve their circulation lifetime (e.g. PEGylation) and their specific cellular targeting (e.g. antibodies). These surface features can give rise to a change in the liposome size and charge.

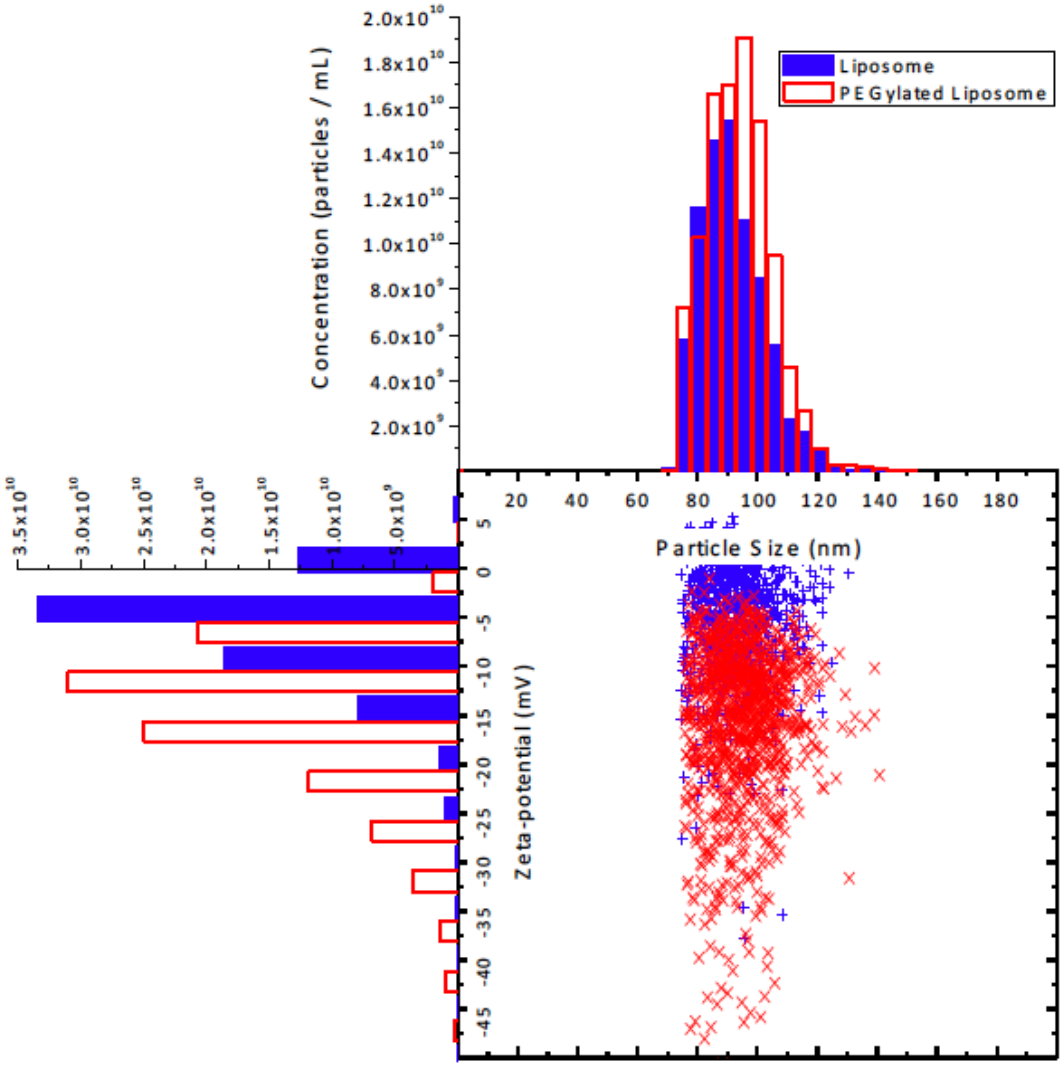


Unmodified Liposome

PEGylated Liposome

PEGylated & Antibody Liposome

Particle-by-Particle Size & Charge Analysis of PEGylated & 'normal' Liposomes



PEGylation – replacing a phosphocholine lipid with a glycolated phospholipid - gives rise to a negative charge.

The degree of liposome PEGylation can be monitored from the corresponding negative shift and distribution in particle zeta-potential.

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Analysis for nanomedicine regulation

- Need for particle by particle sensitive analysis which is accurate, reliable, repeatable and validated
- Size, charge, concentration, interactions
- Leverage current nanotechnology development to improve regulatory outcome and confidence

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Analysis for nanomedicine regulation

- **Comprehensive high resolution analysis to characterize a wide range of nanoparticle systems and individual particle properties**
- **Benefits in assurance for FDA**
- **Benefits in assurance, practicality and cost savings for industry**

Next Step:

- Form project consortium for pilot scheme

- Input from:
  - FDA, EMA
  - Measurement institutes – NIST, NCL, NPL (UK)
  - Nanomedicine companies (4 identified)
  - Izon

Thank You

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